EFFECTS OF INTERMITTENT INJECTIONS OF LHRH ON SECRETORY PATTERNS OF LH AND FSH AND OVARIAN FOLLICULAR GROWTH DURING POSTPARTUM ANOVULATION IN SUCKLED BEEF COWS[1,2]

L. J. Spicer[3], E. M. Convey[4], H. A. Tucker[5] and S. E. Echternkamp[6]

Michigan State University[7]
East Lansing 48824
and
U.S. Department of Agriculture[6]
Clay Center, NE 68933

ABSTRACT

Changes in numbers of ovarian follicles and coincident secretion of pituitary gonadotropins were characterized in suckled, anovulatory beef cows injected iv with 500 ng of luteinizing hormone-releasing hormone (LHRH) every 2 h for 48 or 96 h, starting 21.4 ± .4 d after parturition. Two hours after the last injection, all cows were ovariectomized. Compared with saline-injected controls, LHRH had no effect on baseline or overall concentrations of luteinizing hormone (LH) in serum (P>.10), but increased (P<.05) frequency and decreased (P<.05) amplitude of LH pulses. Luteinizing hormone-releasing hormone increased (P<.05) baseline concentration of follicle stimulating hormone (FSH) in serum and frequency of FSH pulses, but decreased (P<.05) pulse amplitude. Overall concentrations of FSH increased 20% (P<.10). Exogenous LHRH did not affect diameter of the two largest follicles or numbers of follicles 1.0 to 3.9 mm, 4.0 to 7.9 mm or ≥8.0 mm in diameter. These data suggest that increasing the frequency of episodic LH and FSH pulses in postpartum cattle by intermittent administration of LHRH did not increase mean circulating levels of LH, or alter size and numbers of ovarian follicles within the 96-h period of injections. Thus, induction of ovulation in anovulatory cows treated with low-dose injections of LHRH cannot be explained on the basis of an increase in mean concentrations of LH or numbers of antral follicles within 96 h after initiation of injections.

(Key Words: Gonadotropin Releasing Hormone, Gonadotropins, Ovaries, Graafian Follicles, Anestrus, Beef Cattle.)

Introduction

Increased frequency of pulsatile luteinizing hormone (LH) release into blood occurs just before the pre-ovulatory surge of LH in previously anovulatory cows when suckling calves are weaned (Walters et al., 1982a; Faltys et al., 1983). In an attempt to mimic this increase in pulsatile release of LH and thereby induce ovulation, intermittent low doses (500 ng every 2 h) of LH-releasing hormone (LHRH) injected for 4 d to anestrous, suckled (one calf) beef cows successfully induced ovulation in 73% of cows within 8 d post-treatment (Walters et al., 1982b). Five-microgram doses of LHRH given every 2 h for 2 d in beef cows also induced ovulation in four of five cows within 8 d (Riley et al., 1981). Ovulation has also been induced with less than 2.5 μg of LHRH injected every 2 h for 2 to 8 d in anestrous ewes (McLeod et al., 1982a,b) and sows (Cox and Britt, 1982). Presumably, LHRH-induced ovarian stimulation is due to increased frequency of LH and follicle-stimulating hormone (FSH) pulses in serum (Walters et al., 1982b). However, changes in gonadotropin secretory profiles over more than a 4-h period on more than 1 d, or changes in size and numbers of antral follicles, have not been evaluated following multiple, low-dose injections of LHRH. Therefore, the objective of this experiment was to characterize

[1]Michigan Agr. Exp. Sta. Journal Article No. 11695.

[2]We thank Steven Lyth, Lisa Ritchie, Margie McAlhany, Jinnie Stora and Jolene Urbauer for expert technical assistance; K. W. Cheng, Dept. of Physiol., Manitoba, Canada, for supplying bovine FSH and antisera; and the Natl. Pituitary Agency, NIAMDD, for supplying bovine LH.

[3]Present address: M. S. Hershey Medical Center, Pennsylvania State Univ., Hershey, PA 17033.

[4]Present address: Merck, Sharp and Dohme Res. Lab., Merck & Co., Inc., Rahway, NJ 07065.

[5]To whom reprint requests should be addressed.

[6]Roman L. Hruska U. S. Meat Anim. Res. Center.

[7]Dept. of Anim. Sci.

Received September 18, 1985.

Accepted December 17, 1985.

changes in size and numbers of ovarian follicles and coincident secretion of pituitary gonadotropins during postpartum anovulation in suckled beef cows given multiple, low-dose injections of LHRH.

Materials and Methods

Animals and Design. Thirty-eight pluriparous beef cows (26 Angus and 12 Hereford) averaging 6 to 7 yr of age and 400 to 500 kg body weight, were allotted to one of four treatment groups over a 3-wk period beginning on September 14, 1981 at United States Meat Animal Research Center, Clay Center, Nebraska. Two days before start of treatments each week, 10 or 15 cattle were transported from the range, placed in a pen, and fed 11 kg dry matter of supplemented 50:50 corn silage: alfalfa haylage diet (60% total digestible nutrients and 12% protein on a dry matter basis). Throughout treatment each cow was loosely restrained in a stanchion stall in an unheated, enclosed barn and her suckling calf was present. Treatments were begun on d 21.2 ± .4 after parturition and were: 1) injections of saline for 48 h, n = 9; 2) injections of saline for 96 h, n = 10; 3) injections of LHRH for 48 h, n = 9; and 4) injections of LHRH for 96 h, n = 10. Saline (.9% NaCl, 5 ml) and LHRH[8] (500 ng/5 ml saline) were injected iv at 2-h intervals via a jugular cannula. Three Hereford cows were assigned to each of the treatment groups. Cannulas were filled with 50 IU heparin per ml of .9% NaCl between injections to prevent coagulation of blood. Two hours after the last injection all cows were ovariectomized by inserting serrated spay scissors through the dorsal wall of the vagina.

Collection of Serum. Blood (20 ml) was collected via jugular cannula at 15-min intervals for 6 h immediately before ovariectomy. Thus, cattle were bled during the interval of the last three injections. Blood samples were stored for 12 to 20 h at 25 C and centrifuged at 1,500 × g at 4 C for 15 min; sera were decanted, stored at 4 C for an additional 2 to 10 h, then frozen at −20 C until assayed for LH and FSH as described previously (Convey et al., 1976 and Carruthers et al., 1980, respectively).

Follicular Inventory. Immediately after ovariectomy, ovaries were placed in phosphate buffered saline (PBS, pH 7.4) on ice, and the number and size of all follicles 1 mm or greater in diameter on the surface of each ovary were recorded and classified into three sizes: small (1.0 to 3.9 mm), medium (4.0 to 7.9 mm) and large (≥ 8.0 mm), as described previously (Spicer et al., 1986). The two largest follicles from each pair of ovaries were dissected free of ovarian stromal tissue and diameters determined with a vernier caliper.

Statistical Analyses. Six cows (three Angus and three Hereford) were eliminated from statistical analyses once it was determined retrospectively that they had initiated ovulatory cyclicity (as determined by presence of a newly formed corpus luteum or concentrations of progesterone in serum > 1 ng/ml) prior to the onset of treatments (two and three cows in the 48- and 96-h saline-treated groups, respectively and one cow in the 96-h LHRH-treated group). Although numbers of Hereford cows in each group were small, no significant breed effect (Hereford vs Angus) was detectable (P>.10).

Temporal LH and FSH concentrations (expressed as overall mean, baseline, amplitude, height and frequency of secretory pulses) in individual animals were evaluated by an objective method (Spicer et al., 1984). Means of each variable for serum LH and FSH, mean serum progesterone and follicular size and numbers were subjected to a factorial analysis of variance with "LHRH" and "Duration of Injection" as main effects and interactions. Data showing heterogeneous variance were statistically analyzed after transformation to natural log (x + 1). Mean differences were tested using Bonferroni's t-test (Gill, 1978) and Fisher's protected LSD mean test (Ott, 1977) where appropriate.

Results

Follicular Growth. Average diameters (± SE) for largest and second-largest follicles (48 and 96 h pooled) were 11.2 ± .8 and 7.5 ± .6 mm, respectively for controls, and 11.9 ± .5 and 7.3 ± .6, respectively, for LHRH-treated groups (P>.10). Numbers of follicles per pair of ovaries (± SE) in the small, medium and large categories (48 and 96 h pooled) were 24.7 ± 3.9, 11.1 ± 2.5 and 1.8 ± .4 for control cows, and 20.4 ± 4.1, 7.0 ± 1.6 and 1.4 ± .4 for LHRH-treated cows, respectively (P>.10). No newly formed corpora lutea were present at ovariectomy.

[8]Beckman, Palo Alto, CA.

TABLE 1. VARIABLES OF LUTEINIZING HORMONE SECRETION IN ANOVULATORY CATTLE DURING THE LAST 6 H OF EITHER LHRH (L) OR SALINE (S) INJECTIONS FOR 48 OR 96 H[a]

Group	No.	Frequency[b], number/6 h	Baseline, ng/ml	Overall, ng/ml	Amplitude[b], ng/ml (n)	Height[b], ng/ml
48 S	7	1.4 ± .3[c]	.53 ± .10	.94 ± .18[cd]	2.42 ± .54 (10)[c]	2.86 ± .61[c]
48 L	9	3.0 ± .0[d]	.44 ± .06	.72 ± .08[c]	.81 ± .11 (27)[d]	1.25 ± .14[d]
96 S	7	1.7 ± .7[c]	.65 ± .19	1.04 ± .25[cd]	2.69 ± .98 (12)[c]	3.38 ± 1.22[c]
96 L	9	2.9 ± .1[d]	.59 ± .07	1.06 ± .13[d]	1.45 ± .36 (26)[d]	2.05 ± .36[cd]

[a]Mean ± SE; No. = number of animals; (n) = number of pulses.

[b]LHRH effect (P<.01).

[c,d]Means that do not have a common superscript differ (P<.05).

Serum LH. Injections of LHRH did not affect (P>.10) baseline concentrations of LH in serum but increased (P<.01) frequency of pulses of LH from 1.6 per 6 h in controls to 2.9 in LHRH-treated groups (table 1). Although frequency of LH pulses increased, height and amplitude (height minus baseline) of LH pulses decreased (P<.01) after LHRH, which resulted in no change (P>.10) in overall mean concentration of LH in serum. Average amplitude of LH pulses was 2.6 ng/ml in control cows and 1.1 ng/ml in LHRH-treated cows (table 1). The pulsatile pattern of LH release before ovariectomy for one representative cow from control and LHRH-treated groups is depicted in figure 1. A representative cow was defined as one that had a secretory profile closest to the average of the group.

Serum FSH. Frequency of FSH pulses did not differ between control and LHRH-treated

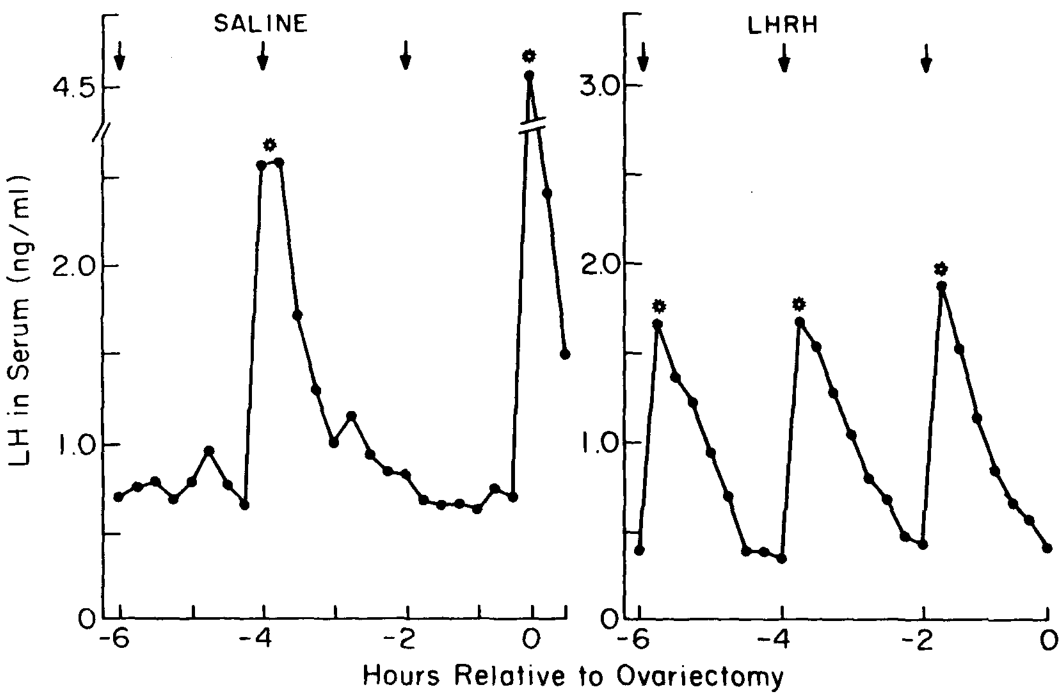

Figure 1. Temporal patterns of LH in serum from representative cows during 6 h before ovariectomy selected from either saline- or LHRH-treated groups. Arrows indicate time of injections and asterisks indicate defined pulses.

TABLE 2. VARIABLES OF FOLLICLE STIMULATING HORMONE SECRETION IN ANOVULATORY CATTLE DURING THE LAST 6 H OF EITHER LHRH (L) OR SALINE (S) INJECTIONS FOR 48 OR 96 H[a]

Group	No.	Frequency[b] number/6 h	Baseline[b], ng/ml	Overall[f], ng/ml	Amplitude[b], ng/ml (n)		Height, ng/ml
48 S	7	$1.6 \pm .3^{c}$	43 ± 2.9^{c}	49 ± 3.5	28 ± 2.4^{cd}	(11)	70 ± 4.3
48 L	9	$1.9 \pm .3^{cd}$	56 ± 5.5^{d}	59 ± 5.5	19 ± 2.2^{e}	(17)	74 ± 5.4
96 S	7	$1.4 \pm .6^{c}$	46 ± 4.4^{cd}	51 ± 3.9	32 ± 4.6^{d}	(10)	75 ± 5.1
96 L	9	$2.5 \pm .4^{d}$	53 ± 4.6^{cd}	59 ± 4.8	22 ± 2.6^{ce}	(23)	75 ± 5.6

[a]Mean ± SE; No. = number of animals; (n) = number of pulses.

[b]LHRH effect (P<.05).

[c,d,e]Means that do not have a common superscript differ (P<.05).

[f]LHRH effect (P<.10).

cows during 42 to 48 h. However, during 90 to 96 h, frequency of FSH pulses was greater in LHRH-treated than control cows (table 2). Injections of LHRH had no effect (P>.10) on height of FSH pulses. Mean baseline of FSH in serum increased 30% (P<.05) and 17% (P<.10) after 42 to 48 h and 90 to 96 h, respectively, and FSH pulse amplitude decreased (P<.05) 32 and 34% after 42 to 48 and 90 to 96 h of LHRH, respectively (table 2). Overall concentrations of FSH were 16 to 21% greater (P<.10) in LHRH-treated than control cows. The pulsatile pattern of FSH release before ovariectomy for one representative cow from control and LHRH-treated groups is depicted in figure 2.

Discussion

An increase in release of LH from one or two pulses per 6 h to three to eight pulses per

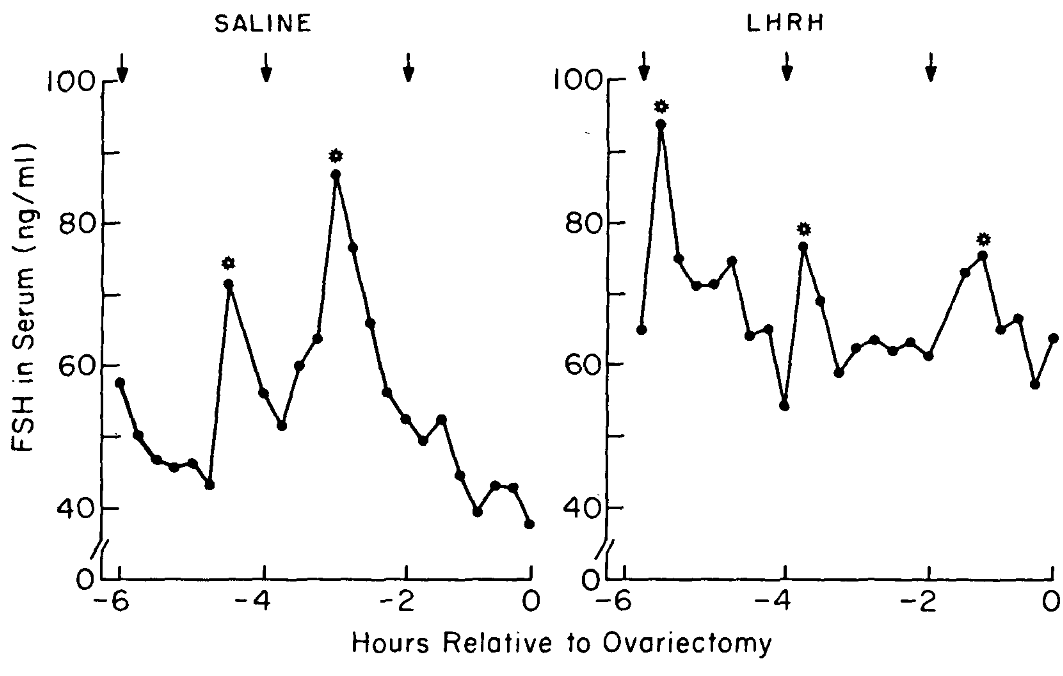

Figure 2. Temporal patterns of FSH in serum from representative cows during 6 h before ovariectomy selected from either saline- or LHRH-treated groups. Arrows indicate time of injections and asterisks indicate defined pulses.

6 h has been suggested to be prerequisite for onset of the first postpartum ovulation in suckled beef (Humphrey et al., 1983) and dairy cows (Peters et al., 1981). Low-dose injections of LHRH at a frequency of three injections per 6 h mimic these pre-ovulatory changes in LH secretion and stimulate ovulation and ovarian cycles in anestrous, suckled beef cows (Riley et al., 1981; Short et al., 1982; Walters et al., 1982b). For example, injections of 500 ng LHRH every 2 h for 96 h subsequently induced ovulation in 8 of 11 suckled beef cows within 8 d (Walters et al., 1982b). Walters et al. (1982b) also observed pre-ovulatory LH surges in 8 of 11 cows to occur during the 4-d treatment period. None of the nine cows injected with LHRH in the present study ovulated during the 4-d injection period. Whether pre-ovulatory LH surges occurred in cattle of the present study cannot be determined since blood was collected only during one 6-h period prior to ovariectomy.

Frequencies of gonadotropin pulses during postpartum anestrus in beef cattle range from 1.2 to 2.2 pulses per 6 h for LH (Walters et al., 1982b; Convey et al., 1983; Humphrey et al., 1983; Williams et al., 1983; Leung et al., 1985) and from 1.2 to 2.8 per 6 h for FSH (Walters et al., 1982b; Convey et al., 1983; Williams et al., 1983). Injections of LHRH given at 2-h intervals nearly doubled these frequencies in the present study and in a previous report (Walters et al., 1982b). In the present study, however, with increased LH and FSH pulse frequency there was a coincident decreased amplitude of gonadotropin pulses; therefore, overall LH concentrations did not change, and overall FSH concentrations increased only 16 to 21%. Previously, identical LHRH treatment regimens increased frequency of LH and FSH pulses without changing LH or FSH pulse height (Walters et al., 1982b). Overall mean concentrations and pulse amplitudes of LH or FSH were not reported by Walters et al. (1982b). Why amplitude of gonadotropin pulses decreased after LHRH injections in the present study is unknown. Perhaps the amount of gonadotropin available to be released from the gonadotrophs (i.e., releasable pools) was limited in the present study (Pickering and Fink, 1979; Evans et al., 1983). In support of this suggestion, Clarke and Cummins (1985) observed that as frequency of exogenous LHRH pulses decreased in ovariectomized ewes in which the pituitary was surgically disconnected from the hypothalamus, the amplitude of the LH responses increased in direct proportion to the size of the releasable LH pool. Depletion of LHRH receptors or some post-receptor mediator are other possibilities (Liu and Jackson, 1984; Leung et al., 1985).

The present study is one of few where overall mean concentrations of FSH in serum increased 20% without a coincident increase in LH concentration. Williams et al. (1983) also observed an increase (29%) in concentrations of FSH in plasma without a change in LH in suckled beef cows between d 7 and 14 postpartum. Pre-ovulatory events before occurrence of gonadotropin surges in cyclic cattle are usually characterized by increased overall concentrations of LH in serum without change in FSH (Padmanabhan et al., 1984; Schallenberger et al., 1985) and may be explained, in part, by increased inhibin production in large follicles (Padmanabhan et al., 1984). Perhaps the control mechanism for pre-ovulatory gonadotropin secretion of anovulatory, postpartum cattle differs from that in cyclic cattle.

Our data suggest that follicular size and numbers are not affected by LHRH-induced changes in gonadotropin secretion within 4 d after initiation of treatment. However, lack of increase in numbers of small follicles should be interpreted carefully because many small follicles could be imbedded in the ovary and not counted.

Numbers of follicles on the surface of ovaries collected between d 20 and 30 after parturition in the present study are similar to numbers reported on d 28 to 42 to 56 after parturition (Spicer et al., 1986). Size of the two largest follicles in the present experiment was also similar to size reported on d 28 to 42 to 56 after parturition (Spicer et al., 1986), but diameters of the largest follicle were smaller than diameters reported for the largest follicle in cattle during estrus (Donaldson and Hansel, 1968; Marion et al., 1968; Merz et al., 1981; Staigmiller et al., 1982). Thus, in the present study, final pre-ovulatory development of the largest follicle was probably not completed.

In summary, both LH and FSH pulse frequency increased coincident with decreased LH and FSH pulse amplitude when LHRH was injected at 2-h intervals for 96 h. The size of the releasable pools of LH and FSH in the gonadotrophs may have been limited in these postpartum anovulatory cattle. Alternatively, decreased sensitivity of the anterior pituitary gland to LHRH or increased inhibition of the response to LHRH may explain the coincident decreases in amplitudes and increases in frequency of gonadotropin pulses. Moreover, the gonadotropin pulses induced by LHRH did not affect follicular growth within the 96-h period studied. Perhaps function other than growth of these follicles are altered by low-dose injections of LHRH.

Literature Cited

Carruthers, T. D., E. M. Convey, J. S. Kesner, H. D. Hafs and K. W. Cheng. 1980. The hypothalamo-pituitary gonadotrophic axis of suckled and nonsuckled dairy cows postpartum. J. Anim. Sci. 51:949.

Clarke, I. J. and J. T. Cummins. 1985. GnRH pulse frequency determines LH pulse amplitude by altering the amount of releasable LH in the pituitary glands of ewes. J. Reprod. Fertil. 73:425.

Convey, E. M., W. E. Beal, E. E. Seguin, K. J. Tannen and Y. C. Lin. 1976. Gonadotropin releasing hormone induced luteinizing hormone release after prostaglandin $F_2\alpha$ in heifers. Proc. Soc. Exp. Biol. Med. 151:84.

Convey, E. M., H. A. Tucker and R. E. Short. 1983. Acute effect of suckling on gonadotropin, prolactin and glucocorticoid concentrations in serum of intact and ovariectomized beef cows. Theriogenology 20:661.

Cox, N. M. and J. H. Britt. 1982. Pulsatile administration of gonadotropin releasing hormone to lactating sows: Endocrine changes associated with induction of fertile estrus. Biol. Reprod. 27:1126.

Donaldson, L. E. and W. Hansel. 1968. Cystic corpora lutea and normal and cystic Graafian follicles in the cow. Australian Vet. J. 44:304.

Evans, W. S., B. J. Boykin, D. L. Kaiser, J. L. C. Borges and M. O. Thorner 1983. Biphasic luteinizing hormone secretion in response to gonadotropin releasing hormone during continuous perifusion of dispersed rat anterior pituitary cells: Changes in total release and the phasic components during the estrous cycle. Endocrinology 112:535.

Faltys, G. L., R. L. Fogwell, R. E. Short and E. M. Convey. 1983. Influence of suckling on secretory patterns of LH and concentrations of cortisol in lactating beef cows. J. Anim. Sci. 57(Suppl. 1):335.

Gill, J. L. 1978. Design and Analysis of Experiments in the Animal and Medical Sciences. Vol. 1. pp. 176-177. Iowa State Univ. Press, Ames.

Humphrey, W. D., C. C. Kaltenbach, T. G. Dunn, D. R. Koritnik and G. D. Niswender. 1983. Characterization of hormonal patterns in the beef cow during postpartum anestrus. J. Anim. Sci. 56:445.

Leung, K., V. Padmanabhan, L. J. Spicer, H. A. Tucker, R. E. Short and E. M. Convey. 1985. Relationship between pituitary GnRH-binding sites and pituitary release of gonadotrophins in post-partum beef cattle. J. Reprod. Fertil. 76:53.

Liu, T. C., and G. L. Jackson. 1984. Long term superfusion of rat anterior pituitary cells: Effects of repeated pulses of gonadotropin-releasing hormone at different doses, durations and frequencies. Endocrinology 115:605.

Marion, G. B., H. T. Gier and J. B. Choudary. 1968. Micromorphology of the bovine ovarian follicular system. J. Anim. Sci. 27:451.

McLeod, B. J., W. Haresign and G. E. Lamming. 1982a. The induction of ovulation and luteal function in seasonally anoestrous ewes treated with small-dose multiple injections of Gn-RH. J. Reprod. Fertil. 65:215.

McLeod, B. J., W. Haresign and G. E. Lamming. 1982b. Response of seasonally anoestrous ewes to small-dose multiple injections of Gn-RH with and without progesterone pretreatment. J. Reprod. Fertil. 65:223.

Merz, E. A., E. R. Hauser and B. G. England. 1981. Ovarian function in the cycling cow: Relationship between gonadotropin binding to the theca and granulosa and steroidogenesis in individual follicles. J. Anim. Sci. 52:1457.

Ott, L. 1977. An Introduction to Statistical Methods and Data Analysis. pp 384-388. Duxbury Press, North Scituate, MA.

Padmanabhan, V., E. M. Convey, J. F. Roche and J. J. Ireland. 1984. Changes in inhibin-like bioactivity in ovulatory and atretic follicles and utero-ovarian venous blood following prostaglandin induces luteolysis in heifers. Endocrinology 115:1332.

Peters, A. R., G. E. Lamming and M. W. Fisher. 1981. A comparision of plasma LH concentrations in milked and suckling post-partum cows. J. Reprod. Fertil. 62:567.

Pickering, A. J.-M. C. and G. Fink. 1979. Variation in size of the "readily releasable pool" of luteinizing hormone during the oestrous cycle of the rat. J. Endocrinol. 83:53.

Riley, G. M., A. R. Peters and G. E. Lamming. 1981. Induction of pulsatile LH release, FSH release and ovulation in postpartum acyclic beef cows by repeated small doses of Gn-RH. J. Reprod. Fertil. 63:559.

Schallenberger, E., A. M. Schondorfer and D. L. Walters. 1985. Gonadotrophins and ovarian steroids in cattle. I. Pulsatile changes on concentrations in the jugular vein throughout the oestrous cycle. Acta Endocrinol. 108:312.

Short, R. E., D. L. Walters, E. M. Convey, R. B. Staigmiller and C. C. Kaltenbach. 1982. Role of pulsatile LH release in resumption of ovarian cycles in postpartum beef cows. In: H. Karg and E. Schallenberger (Ed.) Factors Influencing Fertility in the Postpartum Cow. pp 220-224. Martinus Nijhoff, Boston.

Spicer, L. J., K. Leung, E. M. Convey, J. Gunther, R. E. Short and H. A. Tucker. 1986. Anovulation in postpartum suckled beef cows. I. Associations among size and numbers of ovarian follicles, uterine involution, and hormones in serum and follicular fluid. J. Anim. Sci. 62:734.

Spicer, L. J., K. Sejrsen, H. A. Tucker and J. T. Huber. 1984. Secretion of luteinizing hormone and follicle-stimulating hormone from over-feeding dairy heifers. J. Dairy Sci. 67:1993.

Staigmiller, R. B., B. G. England, R. Webb, R. E. Short and R. A. Bellows. 1982. Estrogen secretion and gonadotropin binding by individual bovine follicles during estrus. J. Anim. Sci. 55:1473.

Walters, D. L., R. E. Short, E. M. Convey, R. B. Staigmiller, T. G. Dunn and C. C. Kaltenbach. 1982a. Pituitary and ovarian function in postpartum beef cows. II. Endocrine changes prior to ovulation in suckled and nonsuckled postpartum cows compared to cycling cows. Biol. Reprod. 26:647.

Walters, D. L., R. E. Short, E. M. Convey, R. B. Staigmiller, T. G. Dunn and C. C. Kaltenbach. 1982b. Pituitary and ovarian function in postpartum beef cows. III. Induction of estrus, ovulation and luteal function with intermittent small-dose injections of GnRH. Biol. Reprod. 26:655.

Williams, G. L., F. Talavera, B. J. Petersen, J. D. Kirsch and J. E. Tilton. 1983. Coincident secretion of follicle-stimulating hormone and luteinizing hormone in early postpartum beef cows: Effects of suckling and low-level increases of systemic progesterone. Biol. Reprod. 29:362.